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PERIODONTOLOGY and PERIODONTICS

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TREATMENT OF FURCATION INVOLVEMENT

The extensions of periodontal pockets that may occur between the roots of multirooted teeth are called furcation involvements.[30] It is important to determine if the involvement has bearing on pulpal pathology,[57,84] since the furcation area has many accessory pulp canals, and if there is pulpal involvement, root canal therapy should precede the periodontal treatment.

There is no difference in basic etiology and pathology between furcation involvements and other periodontal pockets. However, the anatomical and morphological features of the furcations and their relationship to the adjacent structures pose specific problems in treatment of involved teeth.

A long projection of junctional epithelium along enamel projections toward furcations may predispose to pocket formation in this area, although the significance of this anatomical aberration in the development of furcation involvement has never been established.[44] Another common anatomical aberration in the furcation area is enamel pearls and atypical hypoplastic cementum with a very irregular surface morphology (Fig. 25–10). Therefore, if bacterial plaque has gained access after furcation involvement, it is often very difficult to create a root surface which is biologically acceptable to the surrounding soft tissues to an extent that attachment or close adaptation of the surrounding tissue will occur.

From the standpoint of management it may be expedient to divide the furcation involvements into three classes: Class 1 constitutes beginning involvement, not extending more than 2 mm. into the furcation; Class 2 includes involvement extending deeper than 2 mm. but not passing entirely through the furcation; and Class 3 comprises the through and through involvement, in which a probe can be passed between the roots through the entire furcation.

The best treatment for the Class 1 involvement usually is a modified Widman or reverse bevel flap, which will provide access to the area of beginning involvement and remove the epithelial lining of the pocket. The most important part of the treatment is to remove accretions and plane the root surface in such a way that all contaminated cementum is removed. If enamel projections are present, they should be ground off with a fine-pointed diamond stone in order to provide opportunity for connective tissue reattachment. If the furcation is narrow and wedge-shaped a small groove must be created by a fine-pointed diamond stone to ensure complete removal of all contaminated surface cementum as far as the bottom of the furcation. If the crown is bell-shaped, odontoplasty should be done to eliminate part of the furcation undercut by grinding grooves in the buccal or lingual surfaces of the crowns of the teeth extending to the furcation area.[30] If the teeth need restorations, this pattern should be duplicated in the restorations. The less the amount of horizontally directed furcation involvement remaining at the end of the odontoplasty the better will be the periodontal prognosis for the tooth. The flap should be sutured into close approximation to the tooth and held in place by a firm dressing.

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Periodontal repair in dogs: effect of rhBMP-2 concentration on regeneration of alveolar bone and periodontal attachment

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Abstract. The objective of this study was to evaluate the effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) concentration on regeneration of alveolar bone and cementum, and on associated root resorption and ankylosis. Contralateral, critical size, supra-alveolar, periodontal defects were surgically produced and immediately implanted with rhBMP-2 in an absorbable collagen sponge (ACS) carrier in 8, young adult, male, beagle dogs. 6 animals received rhBMP-2/ACS (rhBMP-2 at 0.05, 0.10, or 0.20 mg/mL; total construct volume/defect ~4.0 mL) in contralateral defects following an incomplete block design. 2 animals received rhBMP-2/ACS (rhBMP-2 at 0 and 0.10 mg/mL) in contralateral defects (controls). The animals were euthanised at 8 weeks post-surgery and block sections of the defects were collected for histologic and histometric analysis. Supra-alveolar periodontal defects receiving rhBMP-2 at 0.05, 0.10, or 0.20 mg/ml exhibited extensive alveolar regeneration comprising 86%, 96%, and 88% of the defect height, respectively. Cementum regeneration encompassed 8%, 6%, and 8% of the defect height, respectively. Root resorption was observed for all rhBMP-2 concentrations. Ankylosis was observed in almost all teeth receiving rhBMP-2. Control defects without rhBMP-2 exhibited limited, if any, evidence of alveolar bone and cementum regeneration, root resorption, or ankylosis. Within the selected rhBMP-2 concentration and observation interval, there appear to be no meaningful differences in regeneration of alveolar bone and cementum. There also appear to be no significant differences in the incidence and extent of root resorption and ankylosis, though there may be a positive correlation with rhBMP-2 concentration.

Key words: periodontal defects; dogs; wound healing; growth factors

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rhBMP-2 stimulates clinically significant regeneration of alveolar bone and periodontal attachment in preclinical models (Ishikawa et al. 1994, Sigurdsson et al. 1995, 1996, Kinoshita et al. 1997). The outcome of regeneration, however, appears to be dependent on the operating carrier system.

Using the critical size, supra-alveolar, periodontal defect model, we have observed regeneration of alveolar bone and the periodontal attachment apparatus following implantation of rhBMP-2 using an assortment of candidate carriers (Sigurdsson et al. 1995, 1996). These carriers were canine freeze-dried demineralized bone matrix, bovine deorganified crystalline bone matrix, absorbable type I bovine collagen sponge (ACS), poly(D,L-lactide-co-glycolide) microparticles, and polylactic acid granules. The poly(D,L-lactide-co-glycolide) microparticles exhibited difficult clinical handling. The rhBMP-2/bovine

or canine bone matrix, and the rhBMP-2/polylactic acid constructs were associated with considerable post-surgery swelling. The bovine bone matrix and polylactic acid carrier were associated with compromised histologic bone quality, probably due to the slowly absorbing bone matrix particles or an intense inflammatory reaction caused by the polylactic acid material. Such qualities of a carrier system may limit its clinical utility.

Conversely, rhBMP-2/ACS exhibited advantageous surgical manageability, was associated with minimal clinical swelling, and upon an 8-week healing interval, demonstrated regeneration characterized by dense alveolar bone assuming physiologic form. However, rhBMP-2/ACS constructs appeared to be affected by forces from, or transmitted through, the gingival tissues, which led to apparently limited cementum regeneration, increased ankylosis, and less volume of new bone than desired. The objective of this study was to evaluate the effect of rhBMP-2 concentration (within ACS) on periodontal regeneration. Specifically, regeneration of alveolar bone and cementum, and the incidence and severity of associated root resorption and ankylosis were evaluated in the critical size, supra-alveolar, periodontal defect model following surgical implantation of rhBMP-2/ACS.

Material and Methods

Animals

8 male Beagle dogs, 18 to 24 months old, weight 14 to 16 kg, were used. Animal selection and management, surgical protocol and preparation of periodontal defects followed routines described by Wikesjö et al. (1994) approved by the Institutional Animal Care and Use Committee, Loma Linda University.

The animals were fed a soft consistency laboratory diet (Pedigree®, Kal Kan Foods Inc., Vernon, CA) supplemented with vitamins (Vet A Mix®, Pet-Form®, Vet-A-Mix Inc., Shenandoah, IA) throughout the study. A soft diet was chosen to reduce chance of mechanical interference with healing during food intake.

Before the surgical protocol, the animals had received scaling followed by daily topical application of chlorhexidine (Chlorhexidine Gluconate 20%, ICI Pharmaceutical Group, Wilmington, DE; 20 ml of a 2% solution) to establish healthy gingival conditions.

Surgical protocol

Surgeries were performed under intravenous sodium pentobarbital anesthesia (Nembutal® Sodium Solution, Abbott Laboratories, North Chicago, IL; 20–30 mg/kg IV). The animals were pre-sedated with acepromazine maleate (PromAce®, Aveco Co. Inc., Fort Dodge IA; 1 mg/kg) and received lactated Ringer's solution (Lactated Ringer's Inj., USP, Abbott Laboratories, North Chicago, IL; 100 ml/h IV) during surgery. Routine infiltration anesthesia (Polocaine 2%,1:20,000 levonordefrin, Astra Pharmaceuticals, Westborough, MA) was used at the surgical sites. Sodium thiopental (Pentothal®, Abbott Laboratories, North Chicago, IL; 20–25 mg/kg IV) was used for short-term procedures (i.e., scaling and suture removal).

Defect induction

Critical size, supra-alveolar periodontal defects (Wikesjö et al. 1994) were created around the 3rd and 4th mandibular premolar teeth in right and left mandibular jaw quadrants (Fig. 1). Following sulcular incisions and elevation of buccal and lingual mucoperiosteal flaps, alveolar bone was removed around the full circumference of the teeth including the interproximal and furcation areas with chisels and water-cooled rotating burs. Root surfaces were instrumented with curettes and chisels to remove the cementum (furcation fornices were instrumented with water-cooled rotating diamonds). Clinical defect height was 6 mm from the cemento-enamel junction (CEJ) to the reduced alveolar crest as measured with a probe at the mesial-buccal, buccal, distal-buccal, mesial-lingual, lingual and distal-lingual aspect of the mesial and the distal root. The 1st and 2nd mandibular premolar tooth were extracted and the crown of the 1st molar was amputated level with the reduced alveolar crest.

rhBMP-2 constructs

Lyophilized rhBMP-2 (>98% purity; Genetics Institute Inc., Andover, MA) was reconstituted and diluted in MFR00842 buffer (Genetics Institute Inc., Andover, MA) into concentrations of 0.05, 0.1, and 0.2 mg/ml. For the control, the buffer was used alone.

A sterile ACS (Helistat™, Integra Life Sciences, Plainsboro, NJ), cut to pieces to fit the supra-alveolar periodontal defect, was soak-loaded with 1 of the 4 solutions (0, 0.05, 0.10, or 0.20 mg/ml), and was implanted within 30 min. Total construct volume per defect approximated 4.0 ml.

Wound management

rhBMP-2 and control constructs were fitted around the premolar teeth. Briefly, the soak-loaded construct was piecewise implanted into the furcation and interproximal areas. Rectangular strips, slit-perforated to envelop the teeth, were layered and anchored with additional strips placed cross-wise (Fig. 1). Next, periostea were fenestrated at the base of the flaps and the flap margins coronally advanced to submerge most of the crowns of the teeth (transgingival wound closure) (Fig. 1). Interrupted vertical mattress ties (GORE-TEX Suture CV5, W.L. Gore & Associates Inc., Flagstaff, AZ) were used. Gingival sutures were removed at approximately day 10 post-surgery. Plaque control was maintained by daily topical application of the chlorhexidine solution.

A long-acting opioid (Buprenex Injectable, Buprenorphine HCl, Reckitt & Colman Pharmaceuticals Inc., Richmond, VA; 0.015 mg/kg sc every 12 h for 48 h) was used for immediate post-surgery pain control. A broad spectrum antibiotic (Baytril® Brand of Enrofloxacin, Mobley Corporation, Animal Health Division, Shawnee, KS; 2.5 mg/kg IM every 12 hours for 2 weeks) was used for post-surgery infection control.

Clinical, radiographic and histologic procedures

Photos were taken at defect induction, and at 2, 4, and 8 weeks post-surgery. Radiographs were taken immediately post-surgery, and at 2, 4, and 8 weeks post-surgery. Observations of experimental sites for gingival heålth, wound closure, edema and purulence were made daily in association plaque control procedures and recorded.

The animals were enthanised at week 8 post-surgery by an intravenous injection of concentrated sodium pentobarbital. Tissue blocks including teeth,

bone and soft tissues were removed. The blocks were fixed in 10% buffered formalin for 3 to 5 days, decalcified in 5% formic acid for 8 to 10 weeks, trimmed, dehydrated and embedded in paraffin. Serial sections (7 μm) in a buccal-lingual plane were cut throughout the mesial-distal extension of the teeth. Every 14th section was routine stained with hematoxylin and eosin allowing for observations at 100 μm intervals. Selected additional sections were stained with Ladewig's connective tissue stain modified by Mallory.

Analysis

The most central stained section for each premolar tooth root was identified by the size of the root canal. This section and the immediate stained step serial section on either side were subject to histometric analysis. Thus, 3 subsequent step serial sections, representing approximately 0.2 mm of the mid-portion of each root, were used. Analysis was performed using a Power Macintosh (Apple Computer, Inc., Cupertino, CA) based image analysis system. The following measurements were recorded for the buccal and the lingual surfaces of each root for each section.

- Defect height: distance between apical extension of root planing and CEJ.
- Junctional epithelium: distance from the apical to the coronal extension of the junctional epithelium on the root.
- Connective tissue repair: distance between apical extension of root planing and apical extension of junctional epithelium.
- Cementum regeneration: distance between apical extension of root planing and coronal extension of continuous layer of regenerated cementum or cementum-like deposit.
- Bone regeneration (height): distance between apical extension of root planing and coronal extension of bone regeneration along the root apical to CEJ.
- Bone regeneration (area): cross-sectional area represented by newly formed bone along the root between apical extension of root planing and CEJ.
- Root resorption: combined linear heights of distinct resorption lacunae on the root.
- Ankylosis: combined linear heights of ankylotic union of bone regeneration and the root.

Animal means for each treatment condition were calculated using selected step serial sections. Additionally, frequencies of teeth presenting with cementum regeneration, root resorption and ankylosis were calculated. Presence of these features in 1 or more of the 6 sections for each tooth resulted in a positive score for the tooth. Wound failure (see below) compromising the incomplete block design precluded statistical testing of differences between treatment conditions.

Results

Clinical observations

Surgical implantation of rhBMP-2/ACS or buffer/ACS was uneventful. Wound closure, complicated by the large construct volume, was technically successful for all defects.

Early wound failure, within day 7 post-surgery, was observed in 3 animals with bilateral rhBMP-2/ACS constructs. Bilateral wound failure in 2 animals encompassed horizontal recession of the gingiva exposing instrumented root surfaces. The 3rd animal exhibited unilateral wound failure of similar character. These animals were exited from the study and replaced.

Wound healing was generally uneventful in 4 out of 6 animals completing the study with bilateral rhBMP-2/ACS constructs. The gingival margins receded from their initial coronal position to just coronal to the CEJ with minimal swelling in the post-surgery progression (Fig. 1). Implanted sites appeared hard to palpation at suture removal (approximately day 10 post-surgery). One animal exhibited extensive swelling (soft to palpation) in the left jaw quadrant submerging the teeth; the gingiva exhibiting bluish/purple coloration and a reddish protrusion at the location of the 4th premolar cusp. Eventually the cusp penetrated the tissue that subsequently assumed normal coloration.

2 animals with bilateral rhBMP-2/ACS constructs exhibited unilateral and bilateral wound failure observed from day 14 post-surgery.

Healing appeared similar for the 3 rhBMP-2 concentrations. Wound failure did not correlate to rhBMP-2 concentration.

Healing in the 2 control animals receiving buffer/ACS exhibited gradual gingival recession to circumferentially expose a significant portion of the instrumented root surfaces at week 8 postsurgery (Fig. 2). Contralateral defects receiving rhBMP-2/ACS exhibited healing patterns without complications.

Radiographic observations

Immediately post-surgery, the rhBMP-2/ACS construct was evident as a radiopaque shadow. 2, 4, and 8-week observations revealed increasing radiopacity in all rhBMP-2 implanted jaw quadrants without wound failure. Independent of concentration (0.05, 0.1, or 0.2 mg/ml), 8-week specimens exhibited radiopacity reaching, or ending immediately short of the CEJ (Fig. 3). For the higher concentrations some jaw quadrants presented with radiopacity extending coronal to the CEJ.

At week 8 post-surgery, the radiopaque structures assumed features of trabecular bone. There appeared to be a high variability in the density of the mineralized structure, which was apparently independent of rhBMP-2 concentration. A lamina dura and a periodontal ligament space appeared to have formed in all teeth receiving rhBMP-2/ACS without wound failure.

Limited, if any, regeneration was observed for jaw quadrants exhibiting wound failure within day 14 post-surgery. Radiographically distinct root resorption or ankylosis could not be detected at any observation interval in any of the jaw quadrants. Jaw quadrants receiving buffer/ACS exhibited no suggestion of bone regeneration (Fig. 3). The radiopaque shadow commonly observed immediately post-surgery could not be appreciated in these defects from week 2 post-surgery.

Histologic observations

Irrespective of concentration, all the defects receiving rhBMP-2/ACS exhibited extensive bone regeneration often extending coronal to the CEJ and sometimes covering a portion of the anatomical crown in the buccal-lingual tissue sections (Fig. 4).

At all rhBMP-2 concentrations, numerous well defined trabeculae exhibiting mature physiologic form were apparent. The density and the appearance (woven or lamellar) of newly formed bone varied, as did the size of marrow and fibrovascular spaces, without apparent correlation to rhBMP-2 concentration (Fig. 5). In some cases, bone was observed in contact with enamel.

Cementum regeneration was observed in all specimens but one

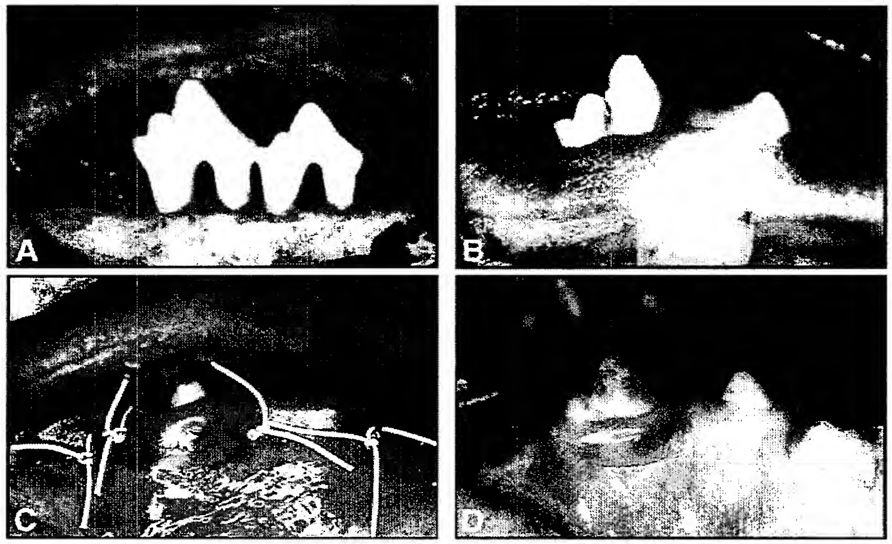

Fig. 1. Critical size, supra-alveolar, periodontal defect implanted with rhBMP-2/ACS. A. Defect preparation. B. Application of the rhBMP-2/ACS implant. C. Wound closure. D. Healing at week 8 post-surgery.

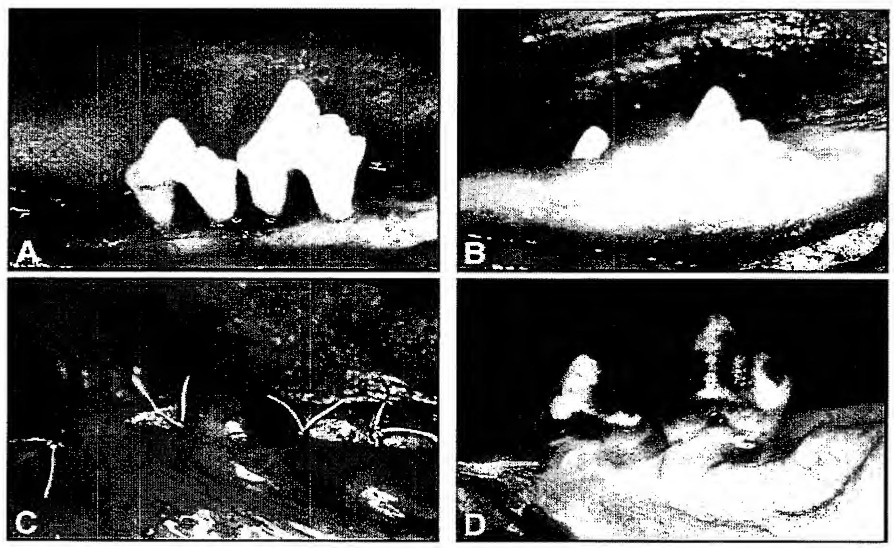

Fig. 2. Critical size, supra-alveolar, periodontal defect implanted with buffer/ACS. A. Defect preparation. B. Application of the buffer/ACS implant. C. Wound closure. D. Healing at week 8 post-surgery.

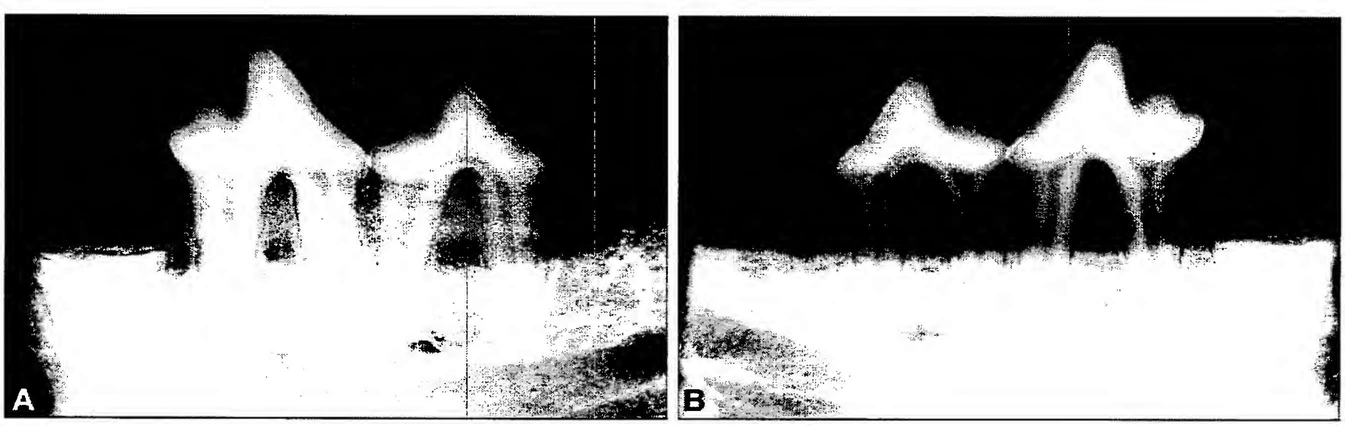

Fig. 3. Radiographic view of the supra-alveolar, periodontal defects in Figs. 1 and 2 at week 8 post-surgery.

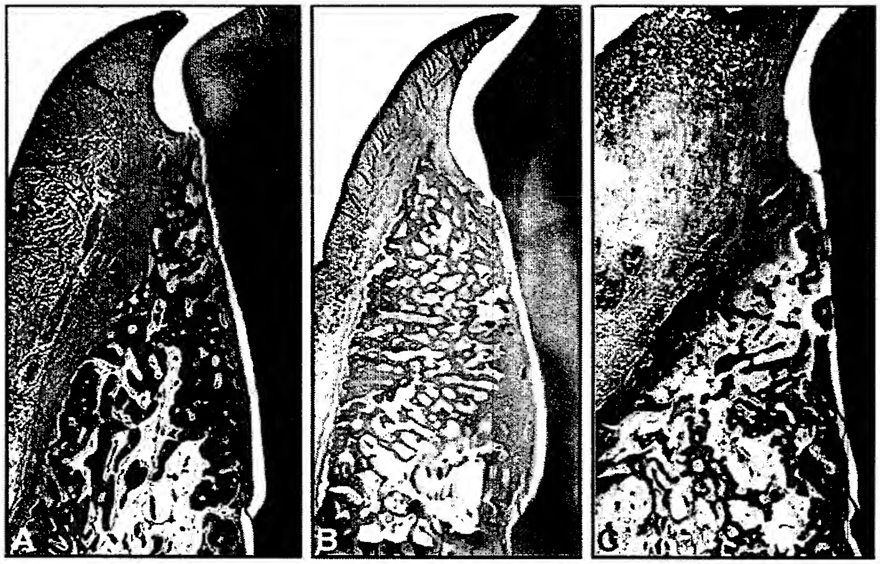

Fig. 4. Photomicrographs of critical size, supra-alveolar, periodontal defects implanted with rhBMP-2/ACS. A. rhBMP-2 at 0.05 mg/ml. B. rhBMP-2 at 0.10 mg/ml. C. rhBMP-2 at 0.20 mg/ml.

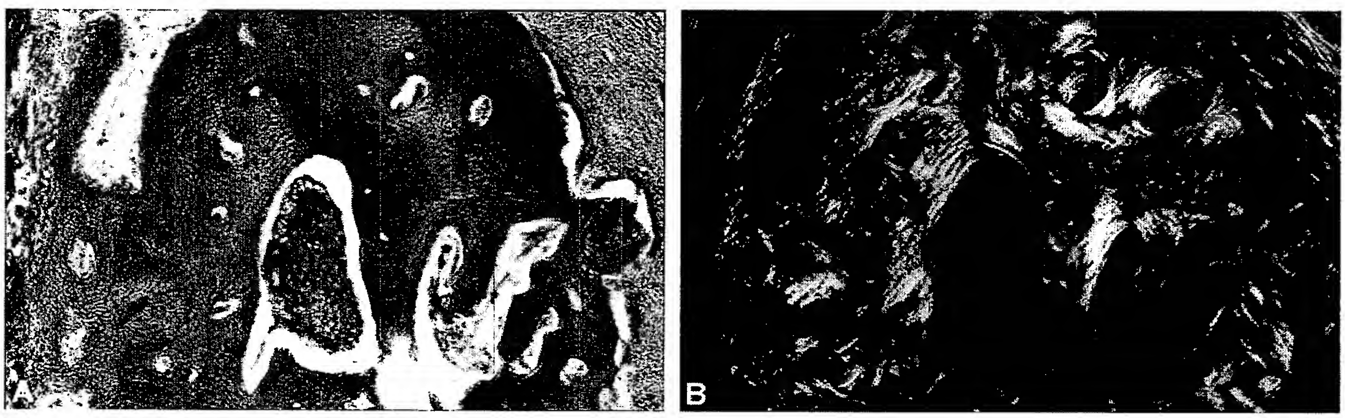

Fig. 5. Photomicrograph of newly formed bone in critical size, supra-alveolar, periodontal defect receiving rhBMP-2 at 0.05 mg/ml. Note the blend of woven and lamellar bone. A. Light microscopy. B. Polarized light microscopy.

(rhBMP-2 at 0.2 mg/ml), and appeared as a continuous cellular layer of varying thickness from the base of the defect, at times becoming indistinguishable from ankylosed bone (Fig. 6). The thickness of the cementum layer did not appear to correlate to rhBMP-2 concentration. A fibrous attachment bridging the gap between newly formed bone and cementum was evident in most specimens.

Ankylosis and root resorption of surface erosion character represented a common observation among the animals without apparent correlation to rhBMP-2 concentration. Animals receiving buffer/ACS, and animals receiving rhBMP-2/ACS who experienced wound failure within day 14 exhibited no or negligible bone and cementum regeneration. Root resorption and ankylosis was not observed in these animals. Rather, the histologic sections presented with epithelization of the flap along the root surface approaching the base of the surgically created defect (Fig. 7). Remnants of the ACS carrier was not observed in any of the histologic sections.

Histometric observations

Tables 1–4 show the results of the histometric analysis. Means and standard deviations are shown for each treatment, for all animals (Table 1), for all the animals excluding jaw quadrants with wound failure (Table 2), and for the controls (Table 3). Table 4 presents frequency of cementum regeneration, root resorption and ankylosis for all animals excluding jaw quadrants with wound failure. Due to the impact of wound failure on the incomplete block design, statistical testing of differences between treatment groups without such healing aberrations was not possible.

Histologic defect height was similar for the various rhBMP-2 and control groups, ranging from 4.9 to 5.4 mm. Generally, connective tissue repair comprised the entire defect height except for defects receiving buffer/ACS, and those exhibiting wound failure, which in part healed with a long junctional epithelium.

Bone regeneration (height) ranged from 4.3 to 5.0 mm for defects without wound failure. The amount of newly formed bone did not appear to correlate to rhBMP-2 concentration. Cementum regeneration was limited and similar for all defects receiving rhBMP-2 (without wound failure), ranging from 0.3 to 0.4 mm. Root resorption ranged between 0.2 and 0.5 mm for defects without wound failure. Root resorption appeared correlated to rhBMP-2 concentration presenting more frequently and in greater extents

Fig. 6. Photomicrographs of critical size, supra-alveolar, periodontal defects implanted with rhBMP-2/ACS. A. Cellular cementum at apical extension of defect. B. Ankylosis at coronal extension of defect.

Fig. 7. Photomicrograph of critical size, supra-alveolar, periodontal defect implanted with buffer/ACS.

Table 1. Histometric measurements, all animals (means±SD (mm))

	0.05 mg/ml	0.10 mg/ml	0.20 mg/ml
defect height	5.0±0.1	5.4±0.4	5.0±0.2
connective tissue repair	4.6±0.8	4.4±1.6	4.2±1.7
cementum regeneration	0.5±0.1	0.3±0.2	0.5±0.2
bone regeneration (height)	3.7±1.1	4.1±1.9	3.6±1.9
bone regeneration (area (mm^2))	9.6±2.2	10.8±4.8	9.4±3.8
root resorption	0.2±0.2	0.4±0.3	0.4±0.3
ankylosis	0.6±0.3	0.9±1.2	1.0±0.8

Table 2. Histometric measurements, excluding jaw quadrants with wound failure (means±SD (mm))

	0.05 mg/ml	0.10 mg/ml	0.20 mg/ml
defect height	5.0±0.1	5.2±0.2	5.1±0.1
connective tissue repair	5.0±0.1	5.2±0.2	5.0±0.1
cementum regeneration	0.4±0.1	0.3±0.2	0.4±0.3
bone regeneration (height)	4.3±0.4	5.0±0.5	4.5±0.4
bone regeneration (area(mm^2)	10.4±1.8	13.1±2.2	11.2±1.3
root resorption	0.2±0.2	0.4±0.4	0.5±0.2
ankylosis	0.7±0.3	1.2±1.3	1.3±0.5

Table 3. Histometric measurements, controls (means±SD (mm))

	0 mg/ml	0.10 mg/ml
defect height	4.9±0.1	5.1±0.5
connective tissue repair	1.5±0.0	5.1±0.5
cementum regeneration	0	1.7±0.1
bone regeneration (height)	0.0±0.1	4.5±0.5
bone regeneration (area (mm^2))	0.2±0.3	12.2±0.5
root resorption	0	0.1±0.1
ankylosis	0	0.3±0.1

in defects receiving rhBMP-2 at 0.2 mg/ml. Ankylosis ranged between 0.7 and 1.3 mm for defects without wound failure. Ankylosis was observed in almost all teeth receiving rhBMP-2. Control defects receiving buffer/ACS exhibited healing dominated by gingival recession and formation of a long junctional epithelium. These defects exhibited limited, if any, bone and cementum regeneration, nor any root resorption nor ankylosis.

Discussion

The objective of this study was to evaluate the effect of rhBMP-2 concentration on regeneration of alveolar bone and cementum, and on associated root resorption and anlylosis. Critical size, supra-alveolar periodontal defects receiving rhBMP-2 at 0.05, 0.10, or 0.20 mg/ml, in an ACS carrier exhibited extensive alveolar regeneration comprising

Table 4. Frequency of teeth with cementum regeneration, root resorption, and ankylosis excluding jaw quadrants with wound failure

	0 mg/ml	0.05 mg/ml	0.1 mg/ml	0.2 mg/ml
cementum regeneration	–	6/6	6/6	5/6
root resorption	–	2/6	4/6	6/6
ankylosis	–	5/6	4/6	6/6
Controls				
cementum regeneration	0/4	–	4/4	–
root resorption	0/4	–	1/4	–
ankylosis	0/4	–	3/4	–

86%, 96%, and 88% of the defect height, respectively, following an 8-week healing interval. Cementum regeneration encompassed 8%, 6%, and 8% of the defect height, respectively. Root resorption appeared more frequently and in greater extents in defects receiving rhBMP-2 at 0.2 mg/ml. Ankylosis was observed in almost all teeth receiving rhBMP-2 and ranged between 14% to 25% of the defect height. Controls receiving buffer/ACS exhibited limited, if any, evidence of alveolar bone and cementum regeneration, root resorption or ankylosis. Within the selected rhBMP-2 concentration and observation interval, there appear to be no meaningful differences in regeneration of alveolar bone and cementum. There also appear to be no meaningful differences in incidence and extent of root resorption and ankylosis.

Discriminating preclinical models provide opportunity to evaluate the efficacy and safety of implants, devices, and biologic constructs aimed at supporting or inducing periodontal regeneration prior to clinical application (Wikesjö et al. 1999). It is essential with any preclinical model considered to evaluate the efficacy of reconstructive therapy in the periodontium, or elsewhere in the skeleton, to satisfy criteria for a critical size defect (Schmitz & Hollinger 1986); i.e., the defect will not regenerate within its lifetime without adjunctive measures. In perspective, the critical size, supra-alveolar, periodontal defect model represents a "litmus test" for the potential of candidate therapies to regenerate alveolar bone and the periodontal attachment apparatus under optimal conditions in a significant biologic environment (Wikesjö et al. 1994, Wikesjö & Selvig 1999). The defect dimensions provide for clinically relevant regeneration of alveolar bone and cementum. The defect morphology allows for an unbiased, appropriate strategy of analysis.

For the analysis of the biologic potential of rhBMP-2 to induce alveolar bone and cementum regeneration, and to influence root resorption and ankylosis, animals (jaw quadrants) exhibiting direct evidence of clinical wound failure were excluded. Although wound closure was technically successful for all defects, 3 animals experienced early wound failure (within day 7 post-surgery). These animals were exited from the study and replaced. Among animals completing the study, 2 animals exhibited "late" wound failure. The wound failures were not correlated to rhBMP-2 concentration. Rather, wound failures, whether manifested early or late, were likely to be related to biomechanical challenges associated with wound closure over the relatively large volume rhBMP-2/ACS construct. This resulted in decreased suture holding strength, suture line rupture, and gingival recession. Since, biomechanical integrity following periodontal reconstructive surgery may not be expected until day 14 post-surgery in dogs (Hiatt et al. 1968), the wound failures must be considered technical failures and should not be included in the evaluation of the biologic potential of rhBMP-2. Unfortunately, the wound failures also compromised the incomplete block design making statistical testing less meaningful. Thus, only summary statistics are provided in this report.

Irrespective of concentration, all defects receiving rhBMP-2 exhibited extensive bone regeneration. The density and appearance (woven or lamellar) of the newly formed bone varied without apparent correlation to rhBMP-2 concentration. In comparison to our previous limited evaluation of rhBMP-2/ACS in supra-alveolar periodontal defects, bone regeneration height was similar and approximated the CEJ in both studies (Sigurdsson et al. 1996). In contrast, bone regeneration area encompassed 10 to 13 mm^2 herein versus 4 mm^2 in our earlier evaluation. Acknowledging that comparisons between studies have limitations even if the studies were performed under nearly identical conditions by the same principal investigator, observed differences in bone regeneration area between studies likely relate to differences in initial construct volume (2.0 versus 4.0 ml). It appears that rhBMP-2/ACS, given sufficient volume, may support relevant regeneration of alveolar bone also in sites where the construct is challenged by a demanding environment such as compression from, or transmitted through, the gingival tissues.

Cementum regeneration was observed as a continuous cellular layer of varying thickness from the base of the defect, oftentimes merging with areas of ankylotic union between the newly formed bone and the root surface. A fibrous attachment bridging the gap between the bone and cementum was evident in most specimens. For the rhBMP-2/ACS treatments, cementum regeneration ranged between 6% to 8% of the defect height and approximated 30% of the defect height in the control animals without apparent correlation to rhBMP-2 concentration. In our previous limited evaluation of rhBMP-2/ACS, cementum regeneration approximated 25% of the defect height (Sigurdsson et al. 1996). The evidence from this study and several preceding histologic studies sustain the premise that rhBMP-2 supports regeneration of the periodontal attachment apparatus including cementum, periodontal ligament, and alveolar bone (Ishikawa et al. 1994, Sigurdsson et al. 1995, 1996, Kinoshita et al. 1997, King et al. 1997, 1998), as has also been suggested for other BMP preparations (Jin 1989, Bowers et al. 1991, Ripamonti et al. 1994, 1996, Giannobile et al. 1998, Kuboki et al. 1998).

Root resorption was a common observation among the animals receiving rhBMP-2/ACS. Root resorption appeared correlated to rhBMP-2 concentration, occurring more frequently and to greater extents in defects implanted with rhBMP-2 at 0.2 mg/ml. However, a strict correlation between root resorption and rhBMP-2 concentration cannot be made from the limited observations in this study. Observed root resorption was mostly of surface erosion character. This is commonly observed in this animal model following periodontal reconstructive surgery includ-

ing guided tissue regeneration and implantation of bone derivatives or substitutes (Sigurdsson et al. 1994, Kim et al. 1998, Wikesjö et al. 1998, Wikesjö & Selvig 1999).

Ankylosis was observed in all groups receiving rhBMP-2/ACS without apparent correlation to rhBMP-2 concentration. The ankylotic union between teeth and newly formed bone was observed in the coronal aspect of the supra-alveolar defect. The cellular cementum extending from the apical extension of the defect oftentimes merged with the ankylotic bone. Similar observations were made for the rhBMP-2/ACS construct and rhBMP-2 in other candidate carriers in our previous study (Sigurdsson et al. 1996). Preceding and parallel studies using rhBMP-2 (Ishikawa et al. 1994, Sigurdsson et al. 1995, 1996, King et al. 1997, 1998) or recombinant human osteogenic protein-1 (rhOP-1/BMP-7) (Ripamonti et al. 1996, Giannobile et al. 1998) in various carriers also provide evidence of ankylosis in large experimental periodontal defects in rodent, canine, and non-human primate models. In the absence of extensive bone regeneration, ankylosis has not been observed (Ripamonti et al. 1996, Kinoshita et al. 1997). In these cases, cementum regeneration with a fibrous attachment has been observed. Similar to the observations herein, varying the concentration of rhOP-1/BMP-7 in large canine furcation defects did not notably influence the extent of ankylosis (Giannobile et al. 1998). It is conceivable that periodontal regeneration following surgical implantation of BMPs may proceed more predictably if bone formation can be somehow controlled. Also, ankylosis may not become a healing aberration in limited periodontal defects since it is rarely observed in the apical aspects of large experimental defects.

Conclusions

rhBMP-2/ACS has significant potential to induce regeneration of alveolar bone. Within the selected rhBMP-2 concentration and observation interval, there appear to be no meaningful differences in regeneration of alveolar bone and cementum. There also appear to be no significant differences in the incidence and extent of root resorption and ankylosis, though there may be a positive correlation with rhBMP-2 concentration.

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Zusammenfassung

Parodontale Reparation bei Hunden: Der Effekt von der rh BMP-2 Konzentration auf die Regeneration des alveolären Knochens und des parodontalen Attachments

Das Ziel der Studie war es, den Effekt der Konzentration des rekombinanten menschlichen knochenmorphogenetischen Proteins-2 (rhBMP-2) auf die Regeneration des alveolären Knochens und des Zementes, auf begleitende Wurzelresorption oder Ankylose zu bestimmen. Bei 8 jungen adulten männlichen Beagle-Hunden wurden kontralateral supraalveoläre parodontale Defekte mit einer kritischen Größe chirurgisch hergestellt und sofort mit rhBMP-2 implantiert, das an resorbierbarem Kollagenschwamm gebunden war (ACS). 6 Tiere erhielten rhBMP-2/ACS (rhBMP-2 zu 0.05, 0.10 oder 0.20 mg/ml; total geschaffenes Volumen/Defekt ~ 4.0 ml) in kontralateralen Defekten, worauf ein unvollständiges Blockdesign folgte. 2 Tiere erhielten rhBMP-2/ACS (rhBMP-2 zu 0 und 0.10 mg/ml) in kontralateralen Defekten (Kontrollen). Die Tiere wurden 8 Wochen postoperativ getötet. Blocksektionen der Defekte für die histologische und histometrische Analyse wurden hergestellt. Die superalveolären parodontalen Defekte, die rhBMP-2 zu 0.05, 0.10 oder 0.20 mg/ml erhielten, zeigten extensive alveoläre Regeneration zu 86%, 95% und 88% der Defekthöhe. Die Zementregeneration betrug 8%, 6% und 8% der Defekthöhe. Wurzelresorptionen wurde für alle rhBMP-2-Konzentrationen beobachtet. Ankylose wurde bei den meisten der Zähne, die rhBMP-2 erhielten, beobachtet. Die Kontrolldefekte ohne rhBMP-2 zeigten limitierte, wenn überhaupt, Zeichen von alveolären Knochen- und Zementregeneration, Wurzelresorption oder Ankylose. Innerhalb der ausgewählten rhBMP-2-Konzentration und Beobachtungsintervalle schien es keine bedeutsamen Differenzen in der Regeneration des alveolären Knochens und des Zementes zu geben. Es gab auch keine signifikanten Differenzen in der Incidenz und Ausdehnung der Wurzelresorption, obwohl es möglicherweise eine positive Korrelation mit der rhBMP-2-Konzentration gibt.

Résumé

Réparation parodontale chez le chien: effet de la concentration en rhBMP-2 sur la régénération de l'os alvéolaire et l'attache parodontale

Le but de cette étude a été d'évaluer l'effet de la concentration de la protéine-2 morphogénétique osseuse humaine reconstituant (rhBMP-2) sur la régénération de l'os alvéolaire et du cément, et sur la résorption radiculaire et l'ankylose associées. Des lésions parodontales contralatérales de grandeur identifiées, supra-alvéolaires ont été créées chirurgicalement et placées immédiatement avec du rhBMP-2 dans un véhicule de Absorbable Collagen Sponge (éponge de collagène absorbable) chez huit jeunes Beagle mâles. Six animaux ont reçu du rhBMP-2/ACS (rhBMP-2 à 0.05, 0.10, ou 0.20 mg/ml; volume total par lésion d'environ 4 ml) dans des lésions contralatérales suivant un modèle de bloc incomplet. 2 animaux ont reçu rhBMP2/ACS(rhBMP-2 à 0 et 0.10 mg/ml) dans des lésions contralatérales (contrôles). Les animaux ont été euthanasiés huit semaines après la chirurgie et des blocs des lésions ont été prélevés pour les analyses histologiques et morphométriques. Les lésions parodontales sus-alvéolaires ayant reçu du rhBMP-2 à 0.05, 0.10 ou 0.20 mg/ml présentaient une régénération alvéolaire importante comprenant respectivement 86%, 96% et 88% de la hauteur de la lésion. La régénération cémentaire était respectivement de 8%, 6% et de 8% de la hauteur de la lésion. La résorption osseuse était observée pour toutes les concentrations de rhBMP-2. L'ankylose a été observée dans presque toutes les dents ayant reçu du rhBMP-2. Les lésions contrôles sans rhBMP-2 montraient peu et parfois pas de régénération osseuse et cémentaire, de résorption radiculaire ou d'ankylose. Dans les concentrations sélectionnées de rhBMP-2 et l'intervalle d'observation suivi il semble n'y avoir aucune différence importante dans la régénération de l'os alvéolaire et le cément. Il semblerait aussi n'y avoir aucune différence dans l'incidence et l'étendue de la résorption radiculaire et de l'ankylose, bien qu'il puisse y avoir une relation positive avec la concentration du rhBMP-2.

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